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Prognostic impact of mitosis and necrosis in non-mucinous lung adenocarcinomas and correlation with IASLC grading system

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Summary. Background. In 2020, the International Lung Cancer Study Group (IASLC) Pathology Committee established a grading system for non-mucinous primary lung adenocarcinomas. This grading system is based on whether areas of high-grade patterns are present in more than 20% of the tumor. Parameters, such as necrosis, mitotic activity, lymphovascular invasion (LVI) and spread through air spaces (STAS), are excluded from evaluating the grading system.

Methods. A total of 217 patients' lung resection materials for primary lung adenocarcinoma were re-reviewed using the IASLC grading system. Necrosis, mitotic activity, LVI status and STAS were also evaluated in the resection materials, aiming to demonstrate the relationship between these histopathological features and clinical outcome data.

Results. At all stages, overall survival (OS) and recurrence-free survival (RFS) were related to grade (p=0.011 and 0.024, respectively). Additionally, patients with necrosis were associated with worse OS and RFS (p=0.002 and 0.048, respectively). When grade 2 and 3 tumors were analyzed individually, a significant relationship was found between necrosis and OS in grade 3 tumors (p=0.002). Patients with a high mitotic count (≥10/10 high-power fields) had significantly worse OS (p=0.046). The prevalence of LVI and STAS increased with grade; however, their prognostic significance has not been demonstrated.

Conclusions. The new grading system provides a highly efficient prognostic classification for survival. Necrosis and high mitotic count are important prognostic parameters for survival. Additionally, necrosis is a stage-independent prognostic factor for OS in grade 3 tumors, although no effect on prognosis can be demonstrated in grade 2 tumors.

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Key words: Lung adenocarcinoma, Grading systems, Necrosis, Mitosis

Introduction

Tumor grading systems are essential in pathologically evaluating solid organ tumors and some hematological malignancies because they provide prognostic information for patients' therapy and management (Elston and Ellis, 1991; Kosary, 1994; Vitolo et al., 2008; Swanson et al., 2021).

Lung adenocarcinomas are malignancies with a broad histomorphological spectrum, characterized by different architectural patterns. There are five major histological architectural patterns: lepidic, acinar, papillary, solid and micropapillary. Recently, cribriform and complex glandular patterns have also been recognized. They coexist in the same tumor at variable rates and combinations depending on the tumor. While the solid, micropapillary, cribriform and complex glandular patterns have a poor prognosis, the papillary and acinar patterns have an intermediate prognosis, and the non-mucinous lepidic pattern has a favorable prognosis (Sica et al., 2010; Yoshizawa et al., 2011; Kadota et al., 2014). For lung adenocarcinomas, various histological grading schemes have been proposed. In 2010, Sica et al. proposed a grading system based on major architectural patterns: low grade, acinar or papillary pattern with a lepidic pattern; intermediate grade, pure acinar/papillary pattern or mixed acinar and papillary pattern or lepidic pattern combined with a solid or micropapillary pattern; high-grade, pure/mixed solid and micropapillary patterns (Sica et al., 2010). According to the World Health Organization (WHO) (2014),

Abbreviations. EGFR, Epidermal Growth Factor Receptor; HPFs, High-power fields; IASLC, International Lung Cancer Working Group; LVI, Lymphovascular invasion; NSCLC, Non-small-cell lung cancer; OS, Overall survival; RFS, Recurrence-free survival; STAS, Spread through air spaces; WHO, World Health Organization

classification was based on the main architectural pattern, as follows: low grade, predominantly lepidic; intermediate grade, predominantly acinar or papillary; high-grade, predominantly solid, micropapillary and cribriform (Travis et al., 2015). The solid, micropapillary and cribriform patterns are ignored if they are not the dominant pattern in this classification (Travis et al., 2015). Because the molecular and intratumoural heterogeneity of lung carcinomas and the treatment response due to this heterogeneity was observed to significantly vary, it was determined that a classification system based on the dominant pattern would be insufficient. As a result, the International Lung Cancer Working Group (IASLC) Pathology Committee classified well-differentiated adenocarcinomas as grade 1 (lepidic predominant pattern and containing <20% high-grade pattern [solid, micropapillary or complex glandular/cribriform]), grade 2 (acinar or papillary adenocarcinomas, with a high-grade pattern <20%) and grade 3 (less differentiated tumors, with high-grade pattern ≥20%) (Moreira et al., 2020). When comparing the prognostic significance of different grading systems, the new IASLC grading system is highly reliable in predicting the outcome of invasive non-mucinous lung adenocarcinoma, regardless of the stage. It also overcomes the limitations of previous grading systems (Lucà et al., 2023). This grading system has been reported to provide more effective results in predicting recurrence-free survival (RFS) and overall survival (OS) than the dominant pattern-based grading system (Rokutan-Kurata et al., 2021; Borczuk, 2022).

Prognosis is consistent, whereas classification is based on the histologically dominant pattern. However, similar to other tumor types, several histological characteristics of lung adenocarcinomas have prognostic significance. Previously used grading systems neglected mitosis, nuclear grade, necrosis, lymphovascular invasion (LVI) and, spread through air spaces (STAS). However, several studies have reported that these parameters are extremely important in predicting prognosis (Kadota et al., 2012; Von der Thüsen et al., 2013; Warth et al., 2017; Liu et al., 2020).

In this study IASLC grade, necrosis, mitotic activity, LVI status and STAS were evaluated in the resection materials, aiming to demonstrate the relationship between these histopathological features and clinical outcome data.

Materials and methods

This study analyzed 217 patients diagnosed with lung adenocarcinoma who underwent surgery at our hospital between 2010 and 2018. Patients with multifocal tumors, those diagnosed with mucinous adenocarcinoma, those with a history of neoadjuvant therapy and those with missing data were excluded from the study. Ethics committee of our institute approved this study (22-10T/16).

Serial sections obtained from all resection materials were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Then, these sections were re-evaluated by two pathologists. The tumors were staged using the 8th edition of the TNM Classification for Lung Cancer (Rami-Porta et al., 2017). The ratios of histological patterns (lepidic, acinar, papillary, micropapillary, solid and complex glandular) observed in each case were recorded with 5% increments.

It was subsequently reclassified using the current IASLC system: grade I (lepidic predominant with <20% high-grade pattern) (Fig. 1A), grade 2 (acinar or papillary predominant with <20% high-grade pattern) (Fig. 1B,C) and grade 3 (any predominant pattern with ≥20% high-grade pattern) (Fig. 1D-I) (9). The presence or absence of necrosis within the tumor was recorded (Fig. 2A,B). The mitotic activity was evaluated as <10 and ≥10 according to the number of mitosis in ten high-power fields (HPFs) (Fig. 2C,D). LVI was defined as present or absent based on the presence of tumor cells in the lymphatic and blood vessels around the tumor (Fig. 2F). The presence of tumor cells (single-cell, micropapillary cluster or solid island) in the air spaces around the main tumor mass was analyzed for STAS (Fig. 2E).

To analyze the Epidermal Growth Factor Receptor (EGFR), DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue blocks using the QIAamp DNA FFPE kit (Qiagen, Germany) based on the kit instructions. Genomic DNA was isolated using the phenol-chloroform method, and specimens with more than 25% tumor cell content were used for the test. Mutations of exons 18, 19, 20, and 21 were analyzed using the therascreen EGFR Pyro Kit on an Applied Rotor-Gene Q 2plex real-time polymerase chain reaction. The mutation analysis of all samples was conducted following the manufacturer's instructions.

Data for normally distributed continuous variables (age) are presented as means and standard deviations, whereas categorical variables are presented as n (%). In comparing variables, the unpaired t-test was used for dependent variables and the chi-square test for categorical variables. The time between the date of surgery and the date of death or the last follow-up was defined as OS. The time between the date of surgery and the date of distant or local recurrence, death or the last follow-up was defined as RFS. The Kaplan-Meier method was used for survival analyses, and the log-rank test was used to compare groups. Cox proportional hazards regression analysis was used for univariate and multivariate analyses of RFS and OS. All analyses were performed using Statistical Package for the Social Sciences, version 24.0. P-values of less than 0.05 were used to denote statistical significance for all analyses.

Results

Clinicopathologic features

In this study, 217 patients were included. The

histopathological characteristics of the patients are shown in Table 1 and Figure 3. The percentages of patients reclassified as grade 1, grade 2 and grade 3 were 8.8% (n=19), 26.2% (n=57) and 65% (n=141), respectively. Of the patients, 163 were male (75.1%) and 54 (24.9%) were female, and the mean age was 61.6±9.3 years. In terms of the predominant pattern, 27 (12.5%) patients had the lepidic predominant pattern, 107 (49.5%) had the acinar or papillary predominant pattern, and 82 (38%) had the high-grade predominant pattern

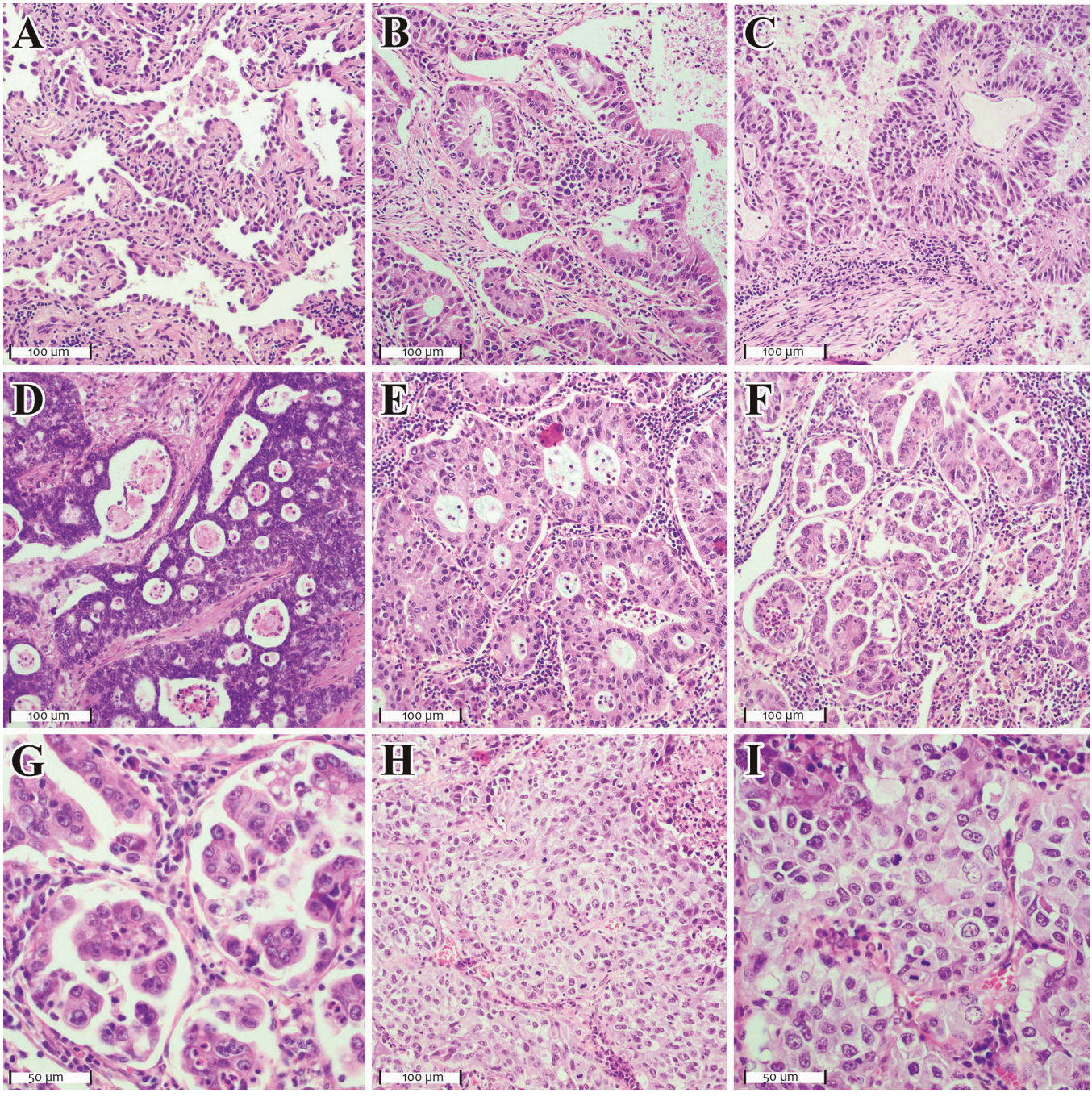

Fig. 1. Different patterns observed in lung adenocarcinomas. Grade 1 tumor with lepidic pattern **(A)**. Grade 2 tumor with acinar pattern **(B)** and with papillary pattern **(C)**. Grade 3 tumor with complex glandular patterns (cribriform **(D)** and fused glands **(E)**). Grade 3 tumor with micropapillary pattern **(F)** and higher magnification from the same case **(G)**. Grade 3 tumor with solid pattern **(H)** and higher magnification from the same case **(I)**. A-F, H, x 200; G, I, x 400.

(solid, micropapillary or complex glandular).

When tumors were graded using the most recent IASLC system, the percentage of male patients increased with the grade (p=0.011). TNM stage, tumor size and nodal stage all increased with the grade (p=0.006, 0.006 and 0.05, respectively). While no necrosis was observed in grade 1 tumors, it was seen in 24.6% of grade 2 tumors and 59.6% of grade 3 tumors (p=0.001). Furthermore, ≥10 mitoses in 10 HPFs were not seen in grade 1 tumors; however, it was observed in 29.8% of grade 2 tumors and 41.6% of grade 3 tumors (p=0.001). LVI and STAS were more common in grade 3 malignancies (p=0.005 and 0.026, respectively).

Survival analysis

In our study, the median follow-up time for all patients was 48 months (range, 4-134 months). During the follow-up period, 81 patients (37.3%) died and 104 (47.9%) relapsed.

For all patients in our study, the mean OS rate was 88.3±3.96 months (95% confidence interval (CI), 80.6-96.1 months), and the mean RFS rate was 75.9±4.01 months) (95% CI, 68.1-83.8 months) in the Kaplan-Meier survival analysis. The mean OS and RFS rates of all patients are shown in Table 2, Table 3 and Figure 4.

At all stages, the grade was correlated with OS; grade 1 tumors had the best prognosis, whereas grade 3 tumors had the worst prognosis (the 5-year OS was 91.7% in patients with grade 1 tumors, 70.8% in patients with grade 2 tumors and 56.3% in patients with grade 3 tumors) (p=0.011). Additionally, a similar correlation was observed between grade and RFS (the 5-year RFS was 64.2% in patients with grade 1 tumors, 53.8% in

Table 1. Relationship Between Clinicopathologic Characteristics and IASLC grades.

Clinicopathologic Characteristics		Total (N=217) n (%)	Grade 1 (N=19) n (%)	Grade 2 (N=57) n (%)	Grade 3 (N=141) n (%)	*p*-Value
Age, y	Mean	61.6	60.6	63.7	60.9	0.136
	SD	9.3	9	9	9.4	
Gender	Male	163 (75.1)	10 (52.6)	39 (68.4)	114 (80.9)	0.011
	Female	54 (24.9)	9 (47.4)	18 (31.6)	27 (19.1)	
Smoking Status	No	62 (28.6)	13 (68.49)	15 (26.3)	34 (24.1)	<0.001
	Yes	155 (71.4)	6 (31.6)	42 (73.7)	107 (75.9)	
p-TNM stage	I	144 (66.4)	18 (94.7)	44 (77.2)	83 (58.2)	0.006
	II	44 (20.3)	1 (5.3)	7 (12.3)	36 (25.5)	
	III	29 (13.4)	0 (0)	6 (10.5)	23 (16.3)	
Tumor stage						
	T1	90 (41.5)	14 (73.7)	28 (49.1)	48 (34.0)	0.006
	T2	98 (45.2)	5 (26.3)	24 (42.1)	69 (48.9)	
	T3	29 (13.3)	0 (0)	5 (8.8)	24 (17.0)	
	T4	0 (0)				
Nodal status	N0	168 (77.4)	18 (94.7)	49 (86.0)	101 (71.6)	0.050
	N1	26 (12.0)	1 (5.3)	3 (5.3)	22 (15.6)	
	N2	23 (10.6)	0 (0)	5 (8.7)	18 (12.8)	
	N3	0 (0)	0 (0)	0 (0)	0 (0)	
Necrosis	Present	98 (45.2)	0 (0)	14 (24.6)	84 (59.6)	<0.001
	Absent	119 (54.8)	19 (100)	43 (75.4)	57 (40.4)	
Mitosis*	<10	141 (65.0)	19 (100)	40 (70.2)	82 (58.2)	0.001
	≥10	76 (35.0)	0 (0)	17 (29.8)	59 (41.8)	
LVI	Present	67 (30.9)	1 (5.3)	13 (22.8)	53 (37.6)	0.005
	Absent	150 (69.1)	18 (94.7)	44 (77.2)	88 (62.4)	
STA	Present	70 (32.3)	1 (5.3)	18 (31.6)	51 (36.2)	0.026
	Absent	147 (67.7)	18 (94.7)	39 (68.4)	90 (63.8)	
Predominant pattern	Lepidic	27 (12.5)	19 (100)	0 (0)	8 (5.7)	<0.001
	Acinar/Papillary	107 (49.3)	0 (0)	57(100)	50 (35.5)	
	High Grade	83 (38.2)	0 (0)	0 (0)	83 (58.8)	
Driver gene mutation	EGFR Wild type	90 (84.9)	5 (62.5)	20 (86.9)	65 (86.7)	0.184
	EGFR Mutation	16 (15.1)	3 (37.5)	3 (13.1)	10 (13.3)	
	Exon 18	4	0	2	2	
	Exon 19 deletion	6	2	1	3	
	Exon 20 insertion	0	0	0	0	
	Exon 21	6	1	0	5	
	EML4-ALK	0	0	0	2	
	N/A	111	11	34	66	

SD, standard deviation; LVI, lymphovascular invasion; STAS, spread through air spaces; N/A, not available. *Number of mitosis in ten high-power fields.

Table 2. Kaplan-Meier overall survival analysis for patients with grade 2, grade 3 and all grades.

Variables	GRADE 2			GRADE 3			ALL GRADES		
	Estimate Mean Survival (Month)	SE (95%CI)	*p* value	Estimate Mean Survival (Month)	SE (95%CI)	*p* value	Estimate Mean Survival (Month)	SE (95%CI)	*p* value
Stage			0.012			0.020			<0.001
I	97.0	6.8 (83.7-110.3)		93.1	6 (81.4-104.9)		100.7	4.4 (92.1-109.2)	
II	51.7	10.6 (31-72.4)		62.8	8.8 (45.5-80.1)		59.2	8.1 (43.4-75.1)	
III	40.5	13.6 (13.9-67.1)		70.3	10.1 (50.5-90.9)		66.5	9.2 (48.6-84.5)	
Necrosis			0.769			0.002			<0.001
Absent	87.0	7.8 (71.7-102.3)		101.3	6.9 (87.8-114.8)		103.9	4.7 (94.6-113.2)	
Present	61.2	5.3 (50.7-71.7)		67.8	6.1 (55.9-79.6)		69.3	5.7 (58.2-80.5)	
Mitosis*			0.874			0.218			0.06
<10	88.9	7.7 (73.8-104)		86.3	6.3 (73.9-98.6)		93.9	4.8 (84.6-103.2)	
≥10	68.0	7.6 (53.2-82.8)		75.6	7.6 (60.7-90.5)		78.7	6.8 (65.5-92)	
LVI			0.063			0.412			0.485
Absent	92.9	6.9 (79.3-106.4)		77.4	6.3 (65-89.89		89.2	4.9 (79.7-98.7)	
Present	55.7	10.3 (35.4-75.9)		86.4	7.8 (71-101.8)		85.4	7.1 (71.4-99.3)	
STAS			0.966			0.552			0.977
Absent	80.2	6.4 (67.7-92.6)		79.3	6.2 (67.2-91.5)		88.9	4.9 (79.4-98.4)	
Present	85.3	11.8 (62.2-108.4)		84.0	8.2 (68-100)		87.2	6.9 (73.7-100.6)	

SE, standard errors; CI, confidence interval; LVI, lymphovascular invasion; STAS, spread through air spaces. *Number of mitosis in ten high-power fields.

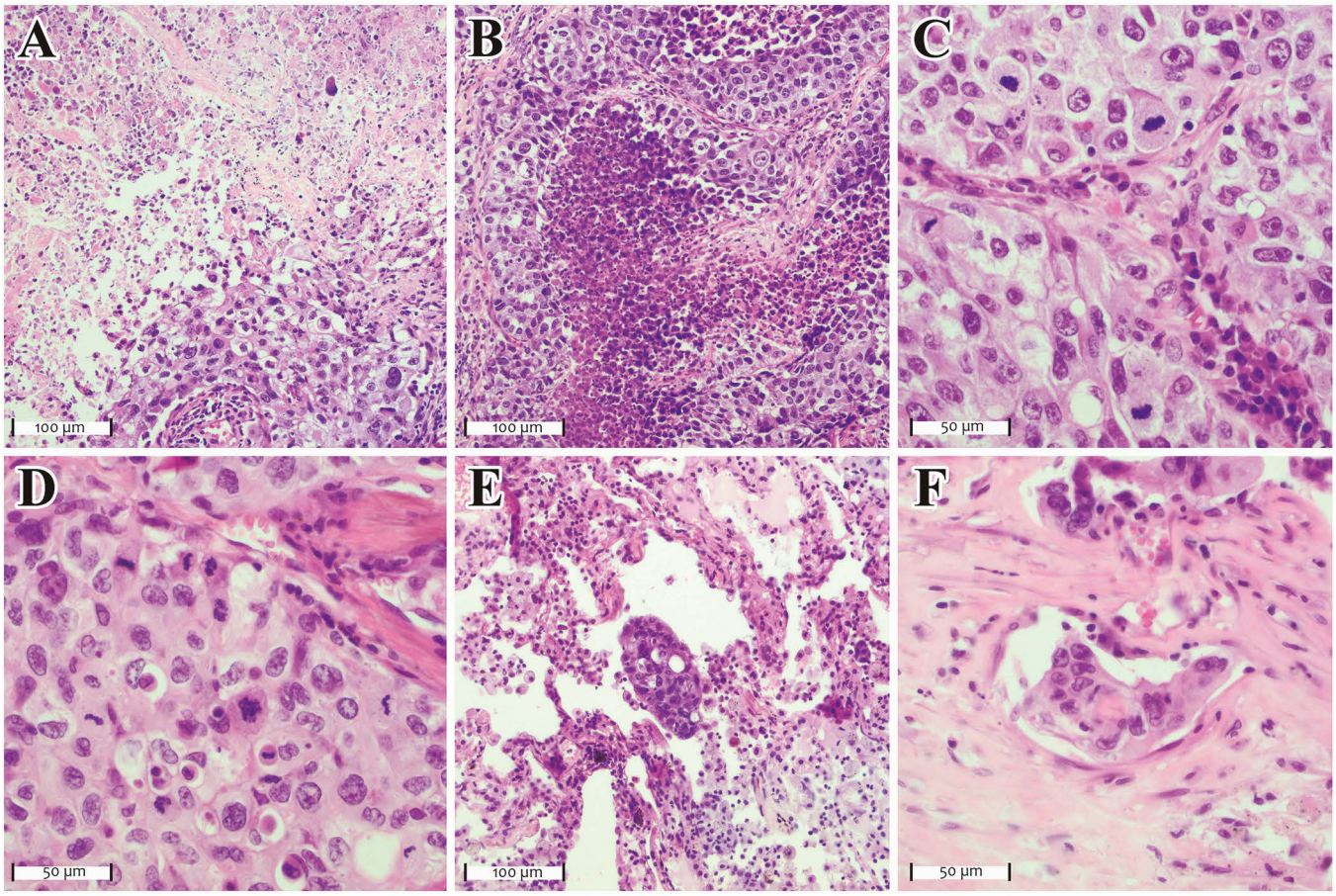

Fig. 2. Examples of necrosis, high mitosis, lymphovascular invasion, and STAS exhibited in adenocarcinomas. **A.** Adenocarcinoma exhibiting extensive necrosis. **B.** Adenocarcinoma displaying central comedo-like necrosis. **C, D.** High mitotic index hotspot area of adenocarcinoma with solid pattern. **E.** Spread through air spaces (STAS). Tumor cluster free floating within air spaces beyond the edge of the tumor. **F.** Lymphovascular invasion (LVI). A, B, E, x 200; C,D, F, x 400.

patients with grade 2 tumors and 46% in patients with grade 3 tumors) (p=0.024).

OS and RFS were significantly better in patients without necrosis (p<0.001 and 0.048, respectively). When grade 2 and 3 tumors were evaluated separately, no correlation was observed between necrosis and OS in grade 2 tumors (p=0.769). In contrast, a more significant correlation was identified in grade 3 tumors (p=0.002). Grade 3 tumors with necrosis had the worst prognosis, whereas the prognosis of grade 3 tumors without necrosis was found to be similar to grade 2 tumors (Fig. 5).

Patients with high mitotic count (≥10/10 HPF) had a poorer OS (p=0.046). The difference was not statistically significant when grade 1 tumors with a very low mitotic count were excluded from the analysis (p=0.874 for grade 2 tumors and p=0.218 for grade 3 tumors). No association was found between mitosis and RFS (p=0.142). Furthermore, the effect of LVI on OS and RFS could not be demonstrated. The 5-year OS was 66.6% in patients without LVI and 64.7% in those with LVI (p=0.282). The 5-year RFS was 51.2% in patients without LVI and 47.1% in those with LVI (p=0.282). No statistically significant relationship was found between STAS and both RFS and OS. The 5-year OS was 61.9% in patients without STAS and 66.1% in those with STAS (p=0.977). The 5-year RFS was 51.2% in patients without STAS and 48.2% in those with STAS (p=0.888).

Table 3. Kaplan-Meier recurrence-free survival analysis for patients with grade 2, grade 3 and all grades.

Variables	GRADE 2			GRADE 3			ALL GRADES		
	Estimate Mean Survival (Month)	SE (95%CI)	*p* value	Estimate Mean Survival (Month)	SE (95%CI)	p value	Estimate Mean Survival (Month)	SE (95%CI)	*p* value
Stage			0.003			0.256			0.004
I	84.8	7.6 (69.9-99.6)		74.5	6.3 (62.2-86.9)		84.1	4.8 (74.8-93.5)	
II	43.9	9.5 (25.3-62.4)		53.3	8.7 (36.2-70.4)		53.2	7.6 (38.3-68.1)	
III	21.5	8.2 (5.5-37.5)		68.5	11.6 (45.8-91.1)		60.8	10.2 (40.8-80.8)	
Necrosis			0.653			0.447			0.048
Absent	78.2	8 (62.6-93.8)		73.6	7.5 (58.9-88.2)		82.4	5.3 (72.9-92.7)	
Present	56.7	9.3 (38.5-74.9)		67.0	6.6 (53.8-79.8)		67.7	6.1 (55.7-79.8)	
Mitosis*			0.926			0.406			0.142
<10	76.8	8.4 (60.4-93.2)		72.9	6.6 (60-85.8)		80.1	5 (70.3-89.9)	
≥10	59.2	8.3 (43.1-75.4)		64.1	7.6 (49.1-79)		68.1	6.9 (55-81.2)	
LVI			0.037			0.663			0.282
Absent	83.1	7.5 (68.4-97.8)		67.2	6.3 (54.9-79.5)		78.2	4.8 (68.8-87.6)	
Present	42.3	10.5 (21.8-62.8)		72.6	8.1 (56.6-88.5)		70.5	7.3 (56.2-84.8)	
STAS			0.959			0.450			0.888
Absent	63.4	6.2 (51.3-75.6)		67.4	6.3 (55-79.8)		75.9	4.94 (66.2-85.6)	
Present	75.9	12.2 (52.-99.7)		73.6	8 (58-89.2)		76.5	6.83 (63.1-89.9)	

SE, standard errors; CI, confidence interval; LVI, lymphovascular invasion; STAS, spread through air spaces. *Number of mitosis in ten high-power fields.

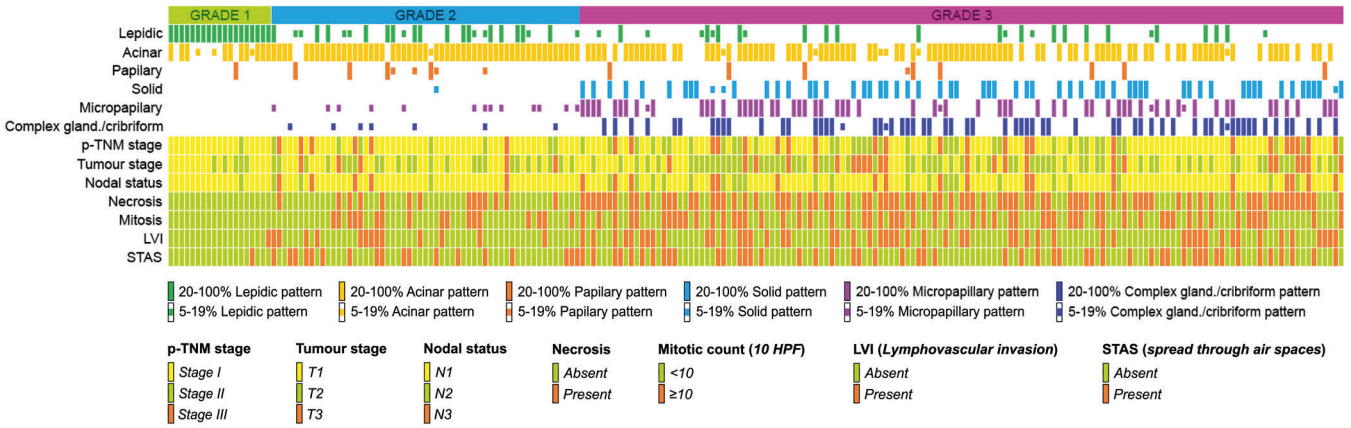

Fig. 3. Clinicopathological features plotted for all cases (each column represents a patient).

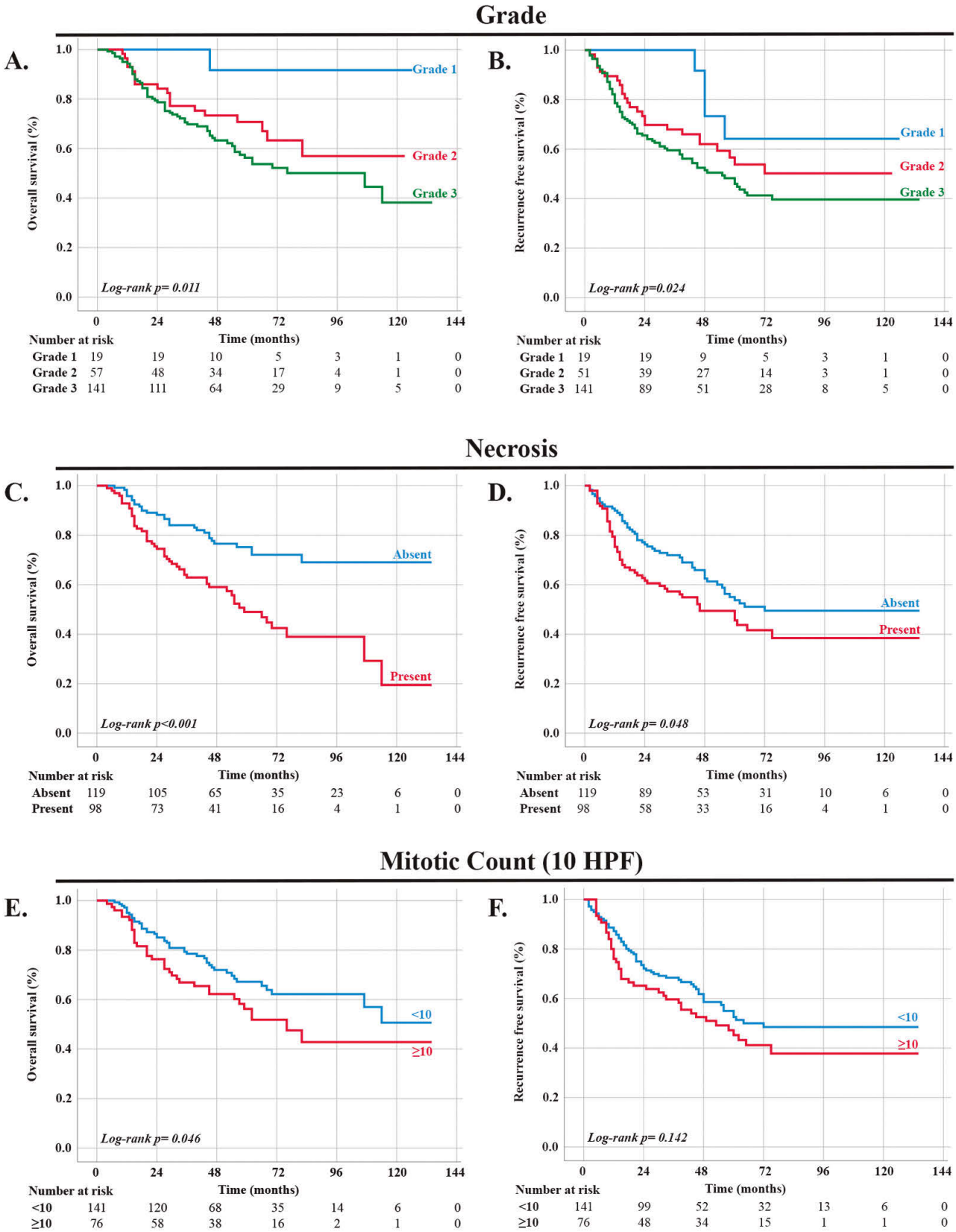

Fig. 4. Kaplan–Meier curves of overall survival and recurrence-free survival for all cases. **A, B.** IASLC grades; **C, D.** Necrosis; **E, F.** Mitosis.

In the univariate analysis, stage II and III tumors (HR=2.773, p=0.002 and HR=2.357, p=0.027, respectively), grade 3 tumors (HR=9.826, p=0.023), necrosis (HR=2.512, p=0.001) and mitosis of ≥10/10 HPF (HR=1.558, p=0.049) had a significantly worse impact on OS among patients with grade 1, 2 and 3 tumors. The multivariate analysis demonstrated that necrosis was an independent prognostic factor for OS (HR=1.762, p=0.045) (Table 4).

Out of all the cases, only 106 could be assessed for EGFR mutation. However, no significant correlation was found between the presence of EGFR mutation and grade (p=0.184) (Table 1). Additionally, there was also no significant correlation found between the presence of

Table 4. Cox regression analysis for overall survival in all cases.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	1.016	0.991-1.042	0.209			
Gender						
Female	1					
Male	2.558	1.353-4.833	0.004	2.230	1.167-4.261	0.015
Grade						
1	1					
2	6.847	0.916-51.160	0.061			
3	9.826	1.362-70.895	0.023			
Stage						
I	1					
II	2.773	1.670-4.604	0.001	2.353	1.382-4.007	0.002
III	2.357	1.320-4.210	0.004	2.133	1.088-4.181	0.027
Necrosis						
Absent	1					
Present	2.512	1.598-3.950	0.001	1.762	1.012-3.068	0.045
Mitosis*						
<10	1					
≥10	1.558	1.002-2.423	0.049	1.375	0.820-2.306	0.227
LVI						
Absent	1					
Present	1.178	0.741-1.872	0.488			
STAS						
Absent	1					
Present	1.007	0.634-1.598	0.978			

HR, hazard ratio; CI, confidence interval; LVI, lymphovascular invasion; STAS, spread through air spaces. *Number of mitosis in ten high-power fields.

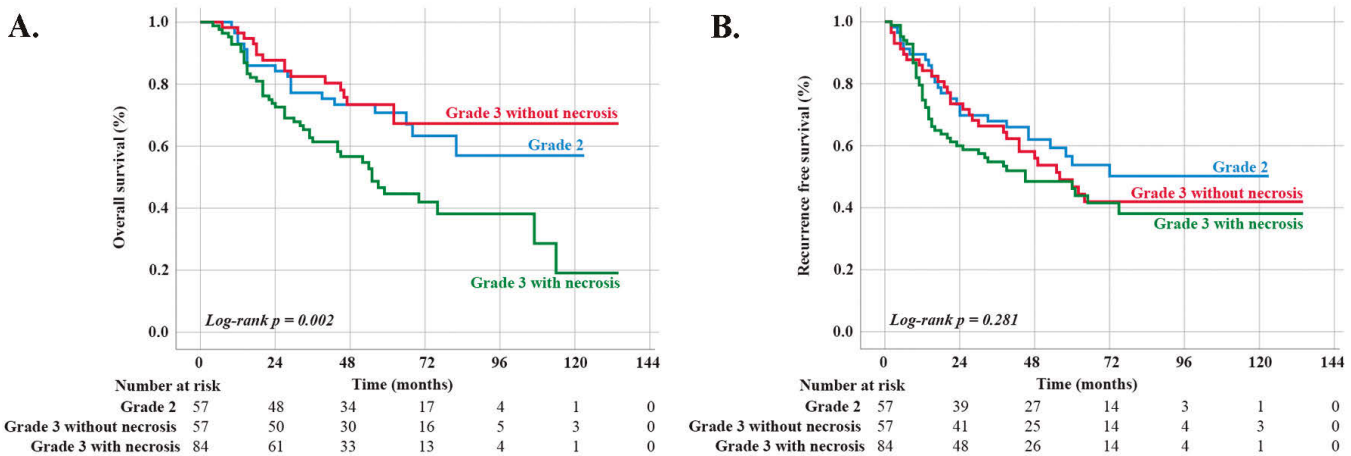

Fig. 5. Comparison of Kaplan-Meier survival curves for grade 3 with necrosis, grade 3 without necrosis and grade 2 cases. **A.** Overall survival. **B.** Recurrence-free survival.

EGFR mutation and OS. Patients without EGFR mutation had a 5-year OS of 58.7%, whereas those with the mutation had a slightly lower rate of 46.5% (p=0.346).

When only stage I patients were analyzed, the 5-year OS was 90.9% in patients with grade 1 tumors, 75.8% in patients with grade 2 tumors and 64.5% in patients with grade 3 tumors (p=0.049). The 5-year RFS was 70.7% in patients with grade 1 tumors, 62.9% in patients with grade 2 tumors and 50.4% in patients with grade 3 tumors (p=0.03). When patients with and without necrosis were analyzed, the 5-year OS in those without necrosis was 79.2%, whereas that in those with necrosis was 55.4% (p=0.005). No statistically significant correlation was found between the presence of necrosis and RFS in stage I patients (the 5-year RFS in patients without necrosis was 58%, whereas that in those with necrosis was 55.5%) (p=0.275). When stage 1 patients were analyzed in terms of mitotic counts, the 5-year OS was 74.7% in patients with <10/10 HPF and 64.9% in those with ≥10/10 HPF with a statistically significant difference (p=0.051). No statistically significant relationship was found between the mitotic count and RFS (the 5-year RFS was 61.8% in patients with <10/10 HPF and 49.7% in those with ≥10/10 HPF) (p=0.079). In stage 1 patients, no statistically significant relationship was observed between LVI status and OS (the 5-year OS was 70.7% in patients without LVI and 72.8% in those with LVI) (p=0.976). Similarly, no statistically significant relationship was found between LVI status and RFS (the 5-year RFS was 58.3% in patients without LVI and 54.6% in those with LVI) (p=0.785). While the 5-year OS in patients without STAS was 71.8%, it was 69.6% in those with STAS, and the difference was not statistically significant (p=0.299). Although the 5-year RFS rates were higher in patients without STAS than in those with STAS (62.7% vs. 48.1%), the difference was not statistically significant (p=0.185).

Discussion

Because lung adenocarcinomas have many variable morphological features, creating an easily applicable and widely recognized morphology-based grading system is more complicated than with other solid tumors. As a result, although various grading systems for lung adenocarcinomas have been suggested, they have yet to gain wide acceptance. However, from this viewpoint, the IASLC Pathology Committee's most recent grading methodology appears promising.

According to studies, the new grading system is more practical and efficient in survival discrimination than previously suggested systems (Deng et al., 2021; Rokutan-Kurata et al., 2021; Hou et al., 2022). In a previous classification system based on the predominant pattern, minor high-grade patterns (solid-micropapillary) and newly defined additional prognosis-related histological patterns (cribriform-fused gland) were not considered. As a result, the prognosis spectrum is broad, and the stratification ability is limited (Yoshizawa et al., 2011; Warth et al., 2012). According to our findings, the new grading system provided an excellent predictive classification for OS and RFS at all stages.

Our data show that grade 3 tumors are more than grade 1 or 2 (even in stage 1 patients). In most studies investigating NSCLC, grade 2 tumors are more than grade 3 tumors in Stage-1 (Rokutan-Kurata et al., 2021; Fujikawa et al., 2022; Woo et al., 2022; Qiu et al., 2023; Yanagawa et al., 2023). However, grade 3 tumors predominate in some studies, consistent with our data (Deng et al., 2021; Xu et al., 2022; Bossé et al., 2023). Furthermore, in these studies, the smoking rate is higher in patients with grade 3 tumors. In our country, and therefore in our study, most cases had a smoking history. The high smoking rate in our study may explain the high number of grade 3 tumors.

Necrosis, LVI, mitosis and STAS are histological markers with prognostic significance other than morphological patterns. The presence of tumor necrosis was an unfavorable risk factor for both OS and RFS in a study of 201 patients with non-small-cell lung cancer (NSCLC), which mainly included patients with lung adenocarcinomas and those with stage 1A tumors only (Park et al., 2011). The presence of necrosis and LVI was a significant independent poor prognostic factor in a study of 485 patients with stage I adenocarcinoma (Kadota et al., 2012). Yoshizawa et al. found that the presence of necrosis in multivariate analysis was an obvious unfavorable prognostic indicator in their study, which included 514 patients with stage I adenocarcinoma (Yoshizawa et al., 2011). Emoto et al. found tumor necrosis in 15% of 1468 cases of stage I lung adenocarcinoma and observed it as a risk factor for recurrence (Emoto et al., 2019). In 2021, Oiwa et al. discovered that tumor necrosis was more common in high-grade patterns in their analysis of 613 patients with stage I lung adenocarcinoma (Oiwa et al., 2021). They found that the presence of necrosis was correlated with a poor prognosis. In this study, the 5-year OS rate of patients with a good prognosis other than the lepidic dominating pattern was 75.5% in the presence of necrosis and 92% in its absence. RFS rates were found to be 59% and 86%, respectively. The presence of vascular invasion and tumor necrosis was found to correlate independently with a higher risk of recurrence in a multivariate analysis. Furthermore, this study found no correlation between the percentage of necrotic area to tumor area and prognosis. In the same study, tumors with and without necrosis were found to have different clinicopathological gene expression characteristics and genetics.

In our study, compatible with the literature, necrosis was observed at a much higher rate in grade 3 tumors than in grade 2 tumors. Patients with necrosis had worse OS and RFS rates, independently of the stage. There were no patients with necrosis in grade 1 tumors when we analyzed the patients separately based on their grades. While no statistically significant difference in

OS and RFS was observed between patients with and without necrosis when considering grade 2 tumors, those with necrosis had considerably worse OS when considering grade 3 tumors. Gkogkou et al. emphasized in their study that the percentage of necrosis is related to the clinical outcome rather than its presence or absence. It was also highlighted that there are differences in the literature regarding the relationship between prognosis and the presence of necrosis. Another cause of the diversity in prognostic data in the literature could be the prevalence of interobserver variability (Gkogkou et al., 2014).

Kadota et al. revealed that the number of mitoses is an important predictor of prognosis. In their studies of stage 1 adenocarcinomas, they discovered that the 5-year RFS decreased as the number of mitoses increased (Kadota et al., 2012). In another study of stage 1 adenocarcinomas, Duhig et al. observed that the mitotic index is an independent prognostic predictor. This difference becomes even more pronounced when the mitotic count is ≥10/10 HPF (Duhig et al, 2015). Our study indicated that OS was worse in patients with mitotic counts ≥10/10 HPF, although it was not an independent prognostic factor. Furthermore, when grade 1 tumors were excluded from the analysis, it was not correlated with OS, and no correlation was found between the number of mitoses and RFS. In Chirieac's study, they recommended both Ki-67 counting and mitotic rate as predictive parameters in NSCLC (Chirieac, 2016). According to Warth et al., Ki-67 is also associated with prognosis in NSCLC (Warth et al, 2014).

Studies in the literature strongly suggest that LVI and STAS are associated with poor prognosis (Yoshizawa et al., 2011; Kadota et al., 2012, 2015; Kato et al., 2012; Warth, 2017; Bains et al., 2019). The incidence of LVI and STAS increases as the grade increases in our study. Even though these two parameters are more common in grade 3 tumors, their association with prognosis could not be determined.

Our study has several limitations. First, as this is a single-center study, the results require to be confirmed by multicenter studies. The second is the limited number of cases analyzed in our study.

In conclusion, it is relatively easier to decide if the high-grade pattern is above 20% than to decide which pattern is dominant. As a result of this inter-observer agreement and practicability for the IASLC grading system were higher than for the conventional predominant pattern-based system. Determining the presence of necrosis is relatively easy, provided adequate tumor sampling and evaluation are performed. Therefore, it is an objective parameter with high interobserver reproducibility. However, further studies are needed to define the appropriate tumor necrosis rate that might influence the prognosis of non-mucinous lung adenocarcinomas.

In addition, we believe that it would be beneficial to include mitotic activity and other proliferative markers that can predict the biological behavior of the tumor in the grading system. In lung adenocarcinomas with high tumor heterogeneity, the prediction of tumor behavior is essential to determine treatment management and response to treatment.

Finally, the new grading system used in our study provided a very excellent prognostic classification for OS and RFS. Furthermore, although its effect on prognosis in grade 2 tumors has not been shown, the presence of necrosis in grade 3 tumors is a prognostic factor independent of the stage for OS.

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Conflict of Interest. All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Ethical Statement. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Ethics Committee of the Ege University (NO.: 22-10T/16) and individual consent for this retrospective analysis was waived.

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